Identification and characterization of plantaricin Q7, a novel plantaricin produced by *Lactobacillus plantarum* Q7

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Abstract

As consumer trends shift toward natural preservatives, it is becoming increasingly necessary to identify antimicrobials that can be used in food products to decrease proliferation of spoilage and pathogenic bacteria. The data presented identifies a novel plantaricin Q7. Lactic acid bacterial strains were isolated from food and of the 118 colonies that were screened, only one produced an inhibitory effect against *Pseudomonas fluorescens* AS 1.1802. Molecular and biochemical methods indicated this bacterium was *Lactobacillus plantarum*. Plantaricin Q7 was purified by absorption-desorption, CM-Fast Flow and Superdex Peptide 10/300 GL. Plantaricin Q7 became inactive following treatment with proteolytic enzyme, including proteinase K, pepsin, trypsin, papain, and pronase E. The plantaricin Q7 remained active following incubation at 121 °C for 20 min and following incubation pH ranging from 3.0-12.0 for 30 min. *Pseudomonas. putida* AS1.1819, *Pseudomonas. aeruginosa* CICC21636 , *Listeria monocytogenes* ATCC19111, *Escherichia coli* ATCC25922, *Salmonella typhimurium* ATCC14028, *Staphylococcus aureus*, *Shigella flexneri* ATCC12022 and *Shigella sonnei* ATCC25931 were all sensitive to plantaricin Q7. The data presented suggests plantaricin Q7 can be applied to food products to inhibit the growth of numerous food spoilage bacteria and pathogens.

Keywords: *Pseudomonas*; *Lactobacillus plantarum*; plantaricin; Characterization

1. Introduction

Psychrophiles are a concern to the dairy industry due to their ability to cause spoilage in raw milk and other dairy products (McClements, Patterson, & Linton, 2001). The ability of psychrophilic bacteria to grow at refrigerated temperatures increases the risk of the pathogen and spoilage bacteria growth during processing and storage. The majority, 67% of psychrophilic bacteria are belong to *Pseudomonas* species (Garcia, Sanz, Garcia-Colia, & Ordonez, 1989). *Pseudomonas* spp. are Gram-negative rods that are considered to be very detrimental organisms because they are responsible for the spoilage of various dairy products and raw milk, due to producing large amounts of extracellular hydrolytic enzymes, mainly lipases and proteases which have thermo-resistant characteristics (Teh et al., 2011). The Gram-negative bacteria, psychrophilic bacteria in raw milk account for 75-99% of the total microbial population, of this population the predominance species were identified as the *Pseudomonas fluorescens* (*Ps. fluorescens*) and *Pseudomonas putida* (*Ps. putida*) (Martins, Pinto, Rocha, De Araujo, & Vanetti, 2006). Although pasteurization kills the *Pseudomonas* spp., the extent of recontamination is a major determinate of their shelf life (Felfoul, Lajnaf, Attia, & Ayadi, 2013). Based on these reports it is evident that elimination of *Pseudomonas* in milk is an important concern from both an agricultural and economic. While preservatives are available which inhibit *Pseudomonas*, consumers are seeking products that minimally processed, have few preservatives and contain natural ingredients. The data presented describes a novel, natural product that can inhibit *Pseudomonas* spp. growth under various conditions.

Antimicrobial peptides isolated from Lactic acid bacteria (LAB) are considered as a bio-preservatives and have been suggested as a potential biological alternative to prevent the propagation of spoilage and pathogenic bacteria in fermented foods (de Jesus Pimentel-Filho, Mantovani, Diez-Gonzalez, & Vanetti, 2013; T Ghrairi, Frere, Berjeaud, & Manai, 2008).

Currently nisin is a natural preservative that is commercially, available and widely used in the dairy

industry (Taoufik Ghrairi, Chaftar, & Hani, 2012). Despite the benefits of nisin, there are some drawbacks, including decreased stability and thus loss of activity at pH range 5.0-7.0 (a common range for dairy products) additionally it is not highly effective on Gram-negative species (Khan, Vu, Riedl, & Lacroix, 2015). As consumer trends shift to less chemical preservatives and more natural ingredients, consideration of economic interests and importantly human health must remain at the forefront during food processing, distribution and storage. While nisin provides necessary foundation, the drawbacks as described above result in limited use, therefore it is essential that alternative bacteriocins that do not have these limitations be developed. The data presented identifies a bacteriocin effective against a broad range of bacteria and the active component is isolated and characterized. The amino acid composition, temperature stability and pH stability were determined and as the data indicate this novel compound is more stable than nisin.

2. Materials and methods

2.1. Isolation of LAB

Samples included cheese from Tibet and XinJiang province, yogurt from Gan Su province, Kumiss from XinJiang province and Yak yogurt from QingHai. These five samples were screened for LAB using the following procedure: One gram cheese or one milliliter liquid samples were prepared in 0.85% sterile saline and 10-fold serial dilutions were prepared. Appropriate dilutions were plated in triplicate on the corresponding selective media. A total volume of 0.1 mL was plated using the spread plate method. M17 agar was used for selection of lactococci, and MRS agar was used for lactobacilli selection. All plates were incubated at 37 °C for 48-72 h. The colonies of catalase negative, Gram-positive were presumed to be LAB. These colonies were sub-cultured onto fresh MRS and stored separately at - 80 °C in MRS broth containing 20% glycerol. All strains subsequently were screened for anti-Psychrophiles bacteriocin.

2.2. Screening anti-Psychrophiles bacteriocin from LAB

All LAB strains, as isolated and stored as described in the previous section, were propagated in MRS at 37 °C for 24 h and had one sub-culture, prior to experimental use. To test for potential bacteriocin activity from these isolated LAB, a 2% inoculum from the sub-culture, was transferred to MRS broth and grown at MRS at 37 °C for 24 h. To screen for potential bacteriocins properties of isolated LAB, the supernatants of LAB was collected from each culture via the following method: Cultures were centrifuged at $8000 \times g$ for 20 min at 4 °C, then the cell-free supernatants (CFS) was collected and adjusted to pH 7.0 with 5 M NaOH, filtered through a 0.22 μm pore-size nitrocellulose membrane. The 96-well diffusion method was used to screen for potential bacteriocin components of LAB. *Ps. fluorescens* was used to test the antimicrobial activity of these potential components. The Oman method (Oman, Boettcher, Wang, Okalibe, & van der Donk, 2011) was utilized, with few modifications, to screen for antimicrobial activity. Sterile petri dish (15 cm) was poured 50 mL solidified agar (1.5 % agar) were prepared as bottom layer. *Ps. fluorescens* AS1.1802 was prepared as indicator bacteria in TSB broth incubated at 30 °C for 12 h. The 50 mL of semi-solid TSB was cooled to 45 °C seeded 10 μL of indicators bacteria (approx 10^8 - 10^9 CFU/mL) and overlaid the solidified agar layer. A sterile 96-well PCR plate was placed in the semi-solid agar and was allowed to solidify at room temperature. After sufficient solidification, the 96-well PCR plate was removed. Each well added into 20 μL and the plates were left at 30 °C for 15 h. The well showed a clear inhibition zone of the indicator strain that were selected as objective strains.

2.3. Characterization of antimicrobial compounds

Pronase E (Sigma-Aldrich, St. Louis, USA) is a protease mixture that results in completely peptide degradation and it was used to categorize the potential bacteriocin as protein (Schadich, Mason, & Cole, 2013). The concentration of pronase E is 1 mg/mL in 20 mM phosphate buffer (pH 7.0). The solution was then added to the CFS (pH 7.0) with final concentration of 100 $\mu g/mL$ and incubation for 30 min at 37 °C. The agar well diffuse assay (AWDA) was used to determine the antimicrobial activity, as described by Khay et al (Khay et al.,

2013). *Ps. fluorescens* AS1.1802 was used as an indicator strains.

2.4. Identification of bacteriocin-producing strain

Gene sequencing of the 16S rDNA was used to identify the bacteriocin-producing strain. Thermocycling parameters were: initial denaturation at 94 °C for 5 min, then 35 cycles of 94 °C for 1 min, 55 °C for 1 min and 3 min at 72 °C for followed by final elongation at 72 °C for 10 min. Universal primers 27F (50-AGAGTTTGATCCTGGCTCAG-30) and 1492R (50-TACGGYTACCTTGTTACGACTT-30) were used for amplification. The PCR products were separated by electrophoresis using 0.8% (w/v) agarose gel and sequenced by Invitrogen (China). The sequences were analyzed and assembled with Vector NTI Advance v11.0 (Invitrogen, Carlsbad, CA, USA). Sequence similarity values between the isolate and related taxa were retrieved from GenBank using BLAST (Basic Local Alignment Search Tool) and the EzTaxon server 2.1 (Jang, Lee, Jung, Choi, & Suh, 2014; Miao et al., 2014). Biochemical analysis was also used the API 50 CHL system to identify strain Q7 (bioMérieux, Marcy l'Etoile, France).

2.5. Purification of plantaricin Q7

Isolation of plantaricin Q7 was from a 2% inoculum into one liter MRS incubated at 37 °C for 24 h. Purification was three-step process. The first step is the absorption-desorption method as previously (Yang, Johnson, & Ray, 1992). Briefly, After 24 h of growth, one liter MRS culture broth was heated to 70 °C for 30 min to kill cells. The pH of the culture broth was adjusted to 6.0 by gradual addition of 5 M NaOH solution. It was stirred for 30 min at room temperature to allow the bacteriocin to adsorb onto the bacterial cells, which were collected by centrifugation at 10,000×g (4°C) for 20 min. To remove debris, the cells were washed twice by resuspension in 100 ml of 5 mM phosphate buffer, recentrifugation at 10,000×g (4°C) and draining off the buffer washings. The cells were resuspended in 100 ml of 0.1 M NaCl solution, and the pH was adjusted to 2.0 by gradual addition of 5% phosphoric acid. The pH 2.0 mixture was stirred in the cold room (4°C) for 1 h,

centrifuged at 12,000×g (4°C) for 20 min and the supernatant fluid was filtered through a 0.22 µm pore-size nitrocellulose membrane. Desorption of the bacteriocin was desalted by using a dialysis membrane (1000 Da cut off) against phosphate buffer (20 mM, pH 6.0). The sample was applied to a CM-Fast Flow cation-exchange chromatography column (length, 100 mm; internal diameter, 16 mm; GE Healthcare, Uppsala, Sweden) equilibrated with 20 mM phosphate buffer, pH 6.0 (buffer A). The bacteriocin was eluted with an increasing NaCl gradient (0 to 1.0 M, linear) in buffer A, at a flow rate of 5 mL/min. The bacteriocin was then detected by UV light at 215 nm. Active fractions were then pooled, dialyzed against distilled water, and then lyophilized. Last step using the Superdex Peptide 10/300 GL (GE Healthcare, Uppsala, Sweden), equilibrated and eluted with 30% acetonitrile contain 0.1% TFA. The bacteriocin detected by UV light at 215 nm, at the flow rate of 0.5 mL/min for 30 min. All peak fractions were tested antibacterial activities by agar well diffusion assay (AWDA). Additionally, total protein concentration of each fraction was determined by an Enhanced BCA Protein Assay Kit.

2.6. High-Performance Liquid Chromatography (HPLC)

Purity of the plantaricin Q7 preparations was evaluated using HPLC. A Jupiter C4 column (250 × 4.6 mm; 5 µm particle size; 300 Å pore size; Phenomenex, Torrance, CA) connected to an Agilent series 1100 HPLC system with in-line degasser, quaternary pump, and diode array detector set to monitor at 215 nm, temperature of the HPLC is room temperature. A 50 µL sample (0.34 mg/mL, concentration from Superdex Peptide 10/300 GL) was loaded onto the column, and active fractions were resolved using a starting mobile phase of 5% acetonitrile in water supplemented with 0.1% trifluoroacetic acid (TFA) and linearly increased to 100% acetonitrile (0.1% TFA) over a 20 min period at a 1 mL/min flow rate.

2.7. Amino acid composition and sequence analysis

The purified peptides were hydrolyzed under vacuum in the presence of constant boiling 6 M HCl for 24 h

at 110 °C in a Pico-Tag station (Water) as previously described (Messaoudi et al., 2012). Following acid hydrolysis, the amino acids were derivatized with Ninhydrin and quantified using RP-HPLC on a Pico-Tag C₁₈ column (3.9 mm × 15 cm) as previous described (Messaoudi et al., 2012). The N-terminal amino acid sequence of plantaricin Q7 was determined by Edman degradation using an ABI Procise 494 Protein Sequence analyzer with an on-line 120A phenylthiohydantoin amino acid analyzer.

2.8. Characterization of plantaricin Q7

Sensitivity of plantaricin Q7 (concentration 1.5 µM, purity is 98%) to various enzymes was tested. The proteases included the proteinase K (0.6 U/mg, pH 6.0, 20 mM phosphate buffer), pepsin (1000U/mg, pH 2, 20 mM acetate buffer), trypsin (1000U/mg, pH 7.0, 20 mM phosphate buffer), papain (17 U/mg, pH 7.0, 20 mM phosphate buffer), and pronase E (1000U/mg, pH 6.0, 20 mM phosphate buffer). All enzymes purchased from Sigma-Aldrich, St. Louis, MO. Samples were denatured by heating them at 100 °C for 5 min. The commercial nisin (concentration 1.5 µM) incubated at the same condition was as positive control.

Temperature stability of the purified plantaricin Q7 (concentration 1.5 µM) was determined as previously described (Luo et al., 2011). The purified sampled was heated at 80 °C, 100 °C 121 °C for 20 min. The commercial nisin (concentration 1.5 µM) was as positive control.

The effect of pH on the activity of purified bacteriocin was tested by adjusting pH to 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0 with HCl (1 M) or NaOH (1 M) and incubated at room temperature for 1 h (T Ghrairi, Frère, Berjeaud, & Manai, 2005). In order to elucidate the possible role of acid pH values in the inhibition of indicators microorganisms, the commercial nisin (concentration 1.5 µM) with adjusted pH at the same values was chosen as positive control. Antimicrobial activity was monitored by using the 96-well diffusion method, *Ps. fluorescens* AS1.1802 as the indicator strain.

2.9. Inhibitory spectrum of plantaricin Q7

The antimicrobial spectrum of the purified plantaricin Q7 (1.5 μ M) was tested. Numerous Gram-positive and Gram-negative species were tested (Table 4). The commercial nisin (concentration 1.5 μ M) was chosen as positive. The indicator strains were inoculated in the appropriate soft agar media and the antimicrobial activities were determined by AWDA.

3. Results

3.1. Isolation LAB and screening of bacteriocin producers

A total of 118 presumptive Gram-positive and catalase-negative LAB strains were isolated from traditional Chinese fermented foods (cheese, Yogurt, Kumiss and Yak yogurt). Only the cell-free fermentation broth of strain Q7 (Yak yogurt from QingHai, China) significantly inhibited *Ps. fluorescens* AS1.1802, at pH 7.0. The cell-free fermentation broth of strain Q7 was treated with pronase E, the antimicrobial activity was disappeared. This data suggests the antimicrobial component confirmed that it is protein substances.

3.2. Identification of the strain Q7

Approximately 1.5 kb fragment of the 16S rDNA was amplified, the gene sequence of strain Q7 showed 98.8% identity to the 16S rDNA of *Lactobacillus plantarum* ATCC14917. This identification was further supported by sugar fermentation reactions using the API 50 CHL system, with a sugar fermentation pattern that 99.9% related to *Lactobacillus plantarum*. Strain Q7 was identified as *Lactobacillus plantarum* (no. KT025850).

3.3. Purification and molecular weight

Plantaricin Q7 was purified by absorption-desorption, CM-Fast Flow and Superdex Peptide 10/300 GL (Table 1, Fig. 1a). The purity of plantaricin Q7 was tested by reverse phase HPLC. Only one peak appeared at 9.40 min (Fig. 1b). The final amount of plantaricin Q7 is 0.34 mg.

3.4. Amino acid composition and sequence analysis

Amino acid composition analyses (Table 2) indicated that plantaricin Q7 contains 40 amino acids, 8 acidic

amnio acids (Asp, Glu), and high proportion of hydrophobic amino acids (14 amino acid), while basic amino acids are 14 (Arg, Lys, His). Interestingly, no cysteine residues were identified, suggesting no disulfides bonds. Additionally, no unusual residues, such as lanthionine, were identified in the purified peptide. Edman degradation was used to determine a partial sequence, residues 1-8 of the plantaricin Q7, and the data indicate that the initial N-terminus sequence is: GHAKIKFD. Based on this limited amino acid sequence, there was no significant homology of the plantaricin Q7 identified in the SWISS-PROT data bank.

3.5. Characterization of Plantaricin Q7

Like nisin, the antimicrobial activity of plantaricin Q7 was inactivated following treatment with all of the proteases tested, confirming a role of protein in the activity. The purified plantaricin Q7 remained active at various physical conditions. These data indicate, unlike commercial nisin, the activity of plantaricin Q7 against *Ps. fluorescens* AS1.1802 is not decreased at pH 3.0, pH 12.0 or exposure to 121°C for 20 min. This data indicate maintained activity of purified plantaricin Q7 over a range of physical components.

3.6. Antimicrobial spectrum of the plantaricin Q7

The data suggest that plantaricin Q7 produced by *Lactobacillus plantarum* Q7 isolated from Yak yogurt has a broad range antimicrobial spectrum. *Ps. fluorescens* AS1.1802 was not the only microbe to demonstrate sensitivity to plantaricin Q7. Various Gram-positive and Gram-negative bacteria also demonstrated sensitivity. Species demonstrated to be sensitive to plantaricin Q7 include several food spoilage bacteria and food-borne pathogens. Specifically, Gram-positive bacteria, *Listeria monocytogenes* ATCC19111, *Staphylococcus aureus*; Gram-negative bacteria including *Escherichia coli* ATCC25922, *Pseudomonas fluorescens* AS1.1802, *Pseudomonas putida* AS1.1819, *Pseudomonas aeruginosa* CICC21636, *Shigella flexneri* ATCC12022, *Shigella sonnei* ATCC25931, *Salmonella enterica* serovar typhimurium ATCC14028. Notably, the commercial nisin only inhibits Gram-positive species.

4. Discussion

The effectiveness of any food preservation techniques are evaluated based on the ability to remove or reduce microorganisms; pathogenic microorganisms need to be reduced to enhance product safety and spoilage microorganisms need to be reduced to improve the shelf life of the food (McClements et al., 2001). Bacteriocins are antimicrobial low molecular weight proteins that are produced by diverse microorganisms including LAB (Cotter, Hill, & Ross, 2005).

Consistent with data reported numerous plantaricins produced by *Lb. plantarum* have been reported previously. For example plantaricin UG1 produced by *Lb. plantarum* UG1 was isolated from dry sausage (Enan, El-Essawy, Uyttendaele, & Debevere, 1996). Two-peptide plantaricin W produced by *Lb. plantarum* PMU33 isolated from som-fak, a Thai low salt fermented fish product (Noonpakdee et al., 2009). Pediocin LB-B1 is an anti-listeria plantaricin produced by *Lb. plantarum* LB-B1, koumiss isolate (Xie et al., 2011). However none of these plantaricins show activity against *Pseudomonas*, a common bacteria involved in food science. It should be noted that two other known plantaricins exhibit activity against the different *Pseudomonas* species: plantaricin MG isolated from “Jiaoke” and plantaricin 163 isolated from Chinese fermented vegetables (Gong, Meng, & Wang, 2010; Hu, Zhao, Zhang, Yu, & Lu, 2013). The data reported presents a novel plantaricin Q7 produced by *Lb. plantarum* Q7 from Yak yogurt, a traditional Chinese fermented milk as well as an extensive description of the compound.

In the procedure of purification, The adsorption-desorption method and cation exchange chromatography have been used extensively for cation antimicrobial peptides purification (Saavedra & Sesma, 2011). Interestingly, many anionic antimicrobial peptides have been reported to be purified by cation exchange column at low pH. For example, the peptide lactocin MXJ32 successfully bound to the cation-exchange column at pH 3.0 (Lü, Yi, Dang, Dang, & Liu, 2014). Additionally, the anionic peptide sakacin B was purified by cation-exchange column

at pH 5.2 (Samelis, Roller, & Metaxopoulos, 1994). Although a high purity plantaricin Q7 was obtained, the final recovery of peptide was quite low. The similar results had been reported by Franz et. al (Franz, Van Belkum, Holzapfel, Abriouel, & Gálvez, 2007). The bacteriocin recovery could be improved by the modification of the culture conditions of producer strain. It was reported that bacteriocin yield was increased effectively when bacteriocin-producing strains were cultured together with an inducer strain or inducer peptide. (Svetoch et al., 2010)

The amino acid compositions of plantaricin Q7 is different from other plantaricin, e.g. ST26MS, ST28MS, bacteriocin AMA-K, plantaricin MG and plantaricin 163 (Gong et al., 2010; Hu et al., 2013; Todorov & Dicks, 2005; Todorov, Nyati, Meincken, & Dicks, 2007). Moreover, the amino acid composition data show two significant differences with other plantaricins. Plantaricin Q7 does not contain cysteine nor tryptophan, which are commonly identified in other plantaricins (Nissen-Meyer, Rogne, Oppegard, Haugen, & Kristiansen, 2009). Additionally, plantaricin Q7 contained more basic amino acids. These unique properties in plantaricin Q7 suggest that its results are interesting because plantaricin Q7 might be a novel antimicrobial peptide and different from the previously reported bacteriocin isolated from another LAB (Drider & Rebuffat, 2011). The further analysis to investigate the structural components of plantaricin Q7, an exact mass, the active domain and gene identification and sequencing will result in bioengineering targets to produce wide range plantaricin Q7.

Many cation plantaricins with a broad antimicrobial spectrum that inhibit not only closely related Lactobacilli but also different foodborne pathogens have also identified, e.g. plantaricin UG1 inhibits various other strains of the genera *Lactobacillus* and *Lactococcus*, in addition to foodborne pathogens including *Listeria monocytogenes*, *Bacillus cereus*, *Clostridium perfringens* and *Clostridium. sporogenes* (Enan et al., 1996). Plantaricin MG and plantaricin 163 showed broad inhibitory activity against Gram-positive and Gram-negative bacteria including *L. monocytogenes*, *S. aureus*, *S.typhimurium*, *E.coli*. and *Pseudomonas* spp. (Gong et al., 2010;

Hu et al., 2013). However, plantaricin Q7 also has the broad antimicrobial spectrum that indicated plantaricin Q7 has different mode of action from cation plantaricins. The further work is research the mode of action of plantaricin Q7. The data presented provides the basis of development of a novel plantaricin to be utilized to decrease food spoilage and transmission of foodborne pathogens.

4. Conclusions

To the best of our knowledge, these results suggest that plantaricin Q7 is a new anti- *Pseudomonas* bacteriocin. The characterization of plantaricin Q7 demonstrated it has potentially to be developed as bio-preservative to control *Pseudomonas* and foodborne pathogen in foods.

Acknowledgements

This work was financially supported by the Doctoral Program of Higher Education of China (No. 20112302110051) and National Science Foundation, China (No. 31271906) and National Science Foundation, China (No. 31571850)

Reference

- Cotter, P. D., Hill, C., & Ross, R. P. (2005). Bacteriocins: developing innate immunity for food. *Nature Reviews Microbiology*, 3(10), 777-788.
- de Jesus Pimentel-Filho, N., Mantovani, H. C., Diez-Gonzalez, F., & Vanetti, M. C. D. (2013). Inhibition of *Listeria* and *Staphylococcus aureus* by bovicin HC5 and nisin combination in milk. *Journal of Agricultural Science*, 5(8), p188.
- Drider, D., & Rebuffat, S. (2011). *Prokaryotic antimicrobial peptides: from genes to applications*: Springer Science & Business Media.
- Enan, G., El-Essawy, A., Uyttendaele, M., & Debevere, J. (1996). Antibacterial activity of *Lactobacillus plantarum* UG1 isolated from dry sausage: characterization, production and bactericidal action of plantaricin UG1. *International Journal of Food Microbiology*, 30(3), 189-215.
- Felfoul, I., Lajnaf, R., Attia, H., & Ayadi, M. A. (2013). Study of Proteolytic and Lipolytic Activities of *Pseudomonas* spp. Isolated From Pasteurized Milk in Tunisia. *Journal of Agricultural Science*, 5(7), p46.
- Franz, C. M., Van Belkum, M. J., Holzapfel, W. H., Abriouel, H., & Gálvez, A. (2007). Diversity of enterococcal bacteriocins and their grouping in a new classification scheme. *FEMS microbiology reviews*, 31(3), 293-310.
- Garcia, M., Sanz, B., Garcia-Colia, P., & Ordonez, J. (1989). Activity and thermostability of the extracellular lipases and proteinases from pseudomonads isolated from raw milk. *Milchwissenschaft*, 44(9), 547-550.
- Ghraiiri, T., Chaftar, N., & Hani, K. (2012). Bacteriocins: recent advances and opportunities. *Progress in Food Preservation*, 485-511.
- Ghraiiri, T., Frère, J., Berjeaud, J., & Manai, M. (2005). Lactococcin MMT24, a novel two-peptide bacteriocin produced by *Lactococcus lactis* isolated from rigouta cheese. *International journal of food microbiology*, 105(3), 389-398.
- Ghraiiri, T., Frere, J., Berjeaud, J., & Manai, M. (2008). Purification and characterisation of bacteriocins produced by *Enterococcus faecium* from Tunisian rigouta cheese. *Food Control*, 19(2), 162-169.
- Gong, H., Meng, X., & Wang, H. (2010). Plantaricin MG active against Gram-negative bacteria produced by *Lactobacillus plantarum* KLDS1. 0391 isolated from “Jiaoke”, a traditional fermented cream from China. *Food control*, 21(1), 89-96.
- Hu, M., Zhao, H., Zhang, C., Yu, J., & Lu, Z. (2013). Purification and characterization of plantaricin 163, a novel bacteriocin produced by *Lactobacillus plantarum* 163 isolated from traditional Chinese fermented vegetables. *Journal of agricultural and food chemistry*, 61(47), 11676-11682.
- Jang, S., Lee, J., Jung, U., Choi, H.-S., & Suh, H. J. (2014). Identification of an anti-listerial domain from *Pediococcus pentosaceus* T1 derived from Kimchi, a traditional fermented vegetable. *Food Control*, 43, 42-48.
- Khan, A., Vu, K. D., Riedl, B., & Lacroix, M. (2015). Optimization of the antimicrobial activity of nisin, Na-EDTA and pH against gram-negative and gram-positive bacteria. *LWT-Food Science and Technology*, 61(1), 124-129.
- Khay, E. O., Idaomar, M., Castro, L., Bernárdez, P., Senhaji, N., & Abrini, J. (2013). Antimicrobial activities of the bacteriocin-like substances produced by lactic acid bacteria isolated from Moroccan dromedary milk. *African Journal of Biotechnology*, 10(51), 10447-10455.
- Lü, X., Yi, L., Dang, J., Dang, Y., & Liu, B. (2014). Purification of novel bacteriocin produced by *Lactobacillus coryniformis* MXJ 32 for inhibiting bacterial foodborne pathogens including antibiotic-resistant microorganisms. *Food Control*, 46, 264-271.

- Luo, F., Feng, S., Sun, Q., Xiang, W., Zhao, J., Zhang, J., & Yang, Z. (2011). Screening for bacteriocin-producing lactic acid bacteria from kurut, a traditional naturally-fermented yak milk from Qinghai–Tibet plateau. *Food Control*, 22(1), 50-53.
- Martins, M. L., Pinto, C. L., Rocha, R. B., De Araujo, E. F., & Vanetti, M. C. (2006). Genetic diversity of Gram-negative, proteolytic, psychrotrophic bacteria isolated from refrigerated raw milk. *International journal of food microbiology*, 111(2), 144-148.
- McClements, J., Patterson, M., & Linton, M. (2001). The effect of growth stage and growth temperature on high hydrostatic pressure inactivation of some psychrotrophic bacteria in milk. *Journal of Food Protection*, 64(4), 514-522.
- Messaoudi, S., Kergourlay, G., Dalgalarondo, M., Choiset, Y., Ferchichi, M., Prévost, H., . . . Dousset, X. (2012). Purification and characterization of a new bacteriocin active against *Campylobacter* produced by *Lactobacillus salivarius* SMXD51. *Food microbiology*, 32(1), 129-134.
- Miao, J., Guo, H., Ou, Y., Liu, G., Fang, X., Liao, Z., . . . Cao, Y. (2014). Purification and characterization of bacteriocin F1, a novel bacteriocin produced by *Lactobacillus paracasei* subsp. *tolerans* FX-6 from Tibetan kefir, a traditional fermented milk from Tibet, China. *Food Control*, 42, 48-53.
- Nissen-Meyer, J., Rogne, P., Oppegard, C., Haugen, H., & Kristiansen, P. (2009). Structure-function relationships of the non-lanthionine-containing peptide (class II) bacteriocins produced by gram-positive bacteria. *Current pharmaceutical biotechnology*, 10(1), 19-37.
- Noonpakdee, W., Jumriangrit, P., Wittayakom, K., Zendo, J., Nakayama, J., Sonomoto, K., & Panyim, S. (2009). Two-peptide bacteriocin from *Lactobacillus plantarum* PMU 33 strain isolated from som-fak, a Thai low salt fermented fish product. *Asia Pasific Journal of Molecular Biology and Biotechnology*, 17(1), 19-25.
- Oman, T. J., Boettcher, J. M., Wang, H., Okalibe, X. N., & van der Donk, W. A. (2011). Sublancin is not a lantibiotic but an S-linked glycopeptide. *Nature chemical biology*, 7(2), 78-80.
- Saavedra, L., & Sesma, F. (2011). Purification techniques of bacteriocins from lactic acid bacteria and other Gram-positive bacteria *Prokaryotic Antimicrobial Peptides* (pp. 99-113): Springer.
- Samelis, J., Roller, S., & Metaxopoulos, J. (1994). Sakacin B, a bacteriocin produced by *Lactobacillus sake* isolated from Greek dry fermented sausages. *Journal of applied bacteriology*, 76(5), 475-486.
- Schadich, E., Mason, D., & Cole, A. L. (2013). Neutralization of bacterial endotoxins by frog antimicrobial peptides. *Microbiology and immunology*, 57(2), 159-161.
- Svetoch, E. A., Eruslanov, B. V., Pereygin, V. V., Levchuk, V. P., Seal, B. S., & Stern, N. J. (2010). Inducer bacteria, unique signal peptides, and low-nutrient media stimulate in vitro bacteriocin production by *Lactobacillus* spp. and *Enterococcus* spp. strains. *Journal of agricultural and food chemistry*, 58(10), 6033-6038.
- Teh, K. H., Flint, S., Palmer, J., Lindsay, D., Andrewes, P., & Bremer, P. (2011). Thermo-resistant enzyme-producing bacteria isolated from the internal surfaces of raw milk tankers. *International Dairy Journal*, 21(10), 742-747.
- Todorov, S., & Dicks, L. (2005). *Lactobacillus plantarum* isolated from molasses produces bacteriocins active against Gram-negative bacteria. *Enzyme and Microbial Technology*, 36(2), 318-326.
- Todorov, S., Nyati, H., Meincken, M., & Dicks, L. (2007). Partial characterization of bacteriocin AMA-K, produced by *Lactobacillus plantarum* AMA-K isolated from naturally fermented milk from Zimbabwe. *Food control*, 18(6), 656-664.
- Xie, Y., An, H., Hao, Y., Qin, Q., Huang, Y., Luo, Y., & Zhang, L. (2011). Characterization of an anti-*Listeria* bacteriocin produced by *Lactobacillus plantarum* LB-B1 isolated from koumiss, a traditionally fermented dairy product from China. *Food control*, 22(7), 1027-1031.

Yang, R., Johnson, M. C., & Ray, B. (1992). Novel method to extract large amounts of bacteriocins from lactic acid bacteria. *Applied and Environmental Microbiology*, 58(10), 3355-3359.

399 Table 1 Purification of plantaricin Q7 produced by *Lactobacillus plantarum* Q7

Step	Total activity (AU) ^a	Total protein (mg) ^b	Specific activity (AU mg ⁻¹) ^c	Yield (%)	Purification (fold)
Supernatant	2.4×10^5	1.4×10^4	16.8	100	1
Adsorb-desorb	1.9×10^5	4.1×10^3	46	80	2.8
CM-FF	6.5×10^4	0.71×10^3	92	63	5.5
Superdex Peptide	2.4×10^4	0.34	7.1×10^4	10	4202

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416 **Table 2 Amino acid composition of planteriocin Q7**

Amino acids	Residues per molecule ^a
Asp	4
Thr	2
Ser	2
Glu	4
Gly	4
Ala	2
Cys	0
Val	2
Met	0
Ile	1
Leu	2
Tyr	0
Phe	2
Lys	7
His	2
Arg	5
Pro	1

417 ^a Mean values from three runs. The values in parentheses are the numbers of the individual amino acids in
 418 planteriocin Q7.

419

Figure Caption

Fig. 1 (a) Separation of plantaricin Q7 by Superdex Peptide 10/300 GL. (b) Purity of plantaricin Q7 identified by HPLC.

442 **Fig. 1**